THE HENRY SHAW SCHOOL

March 15, 1955

Dr. Rosalind E. Franklin Birkbeck College University of London 21 Torrington Square, W.C.1. London England

Dear Dr. Franklin:

I have gone over the material which you send me with a great deal of interest. Your investigations certainly promise to tell us a great deal of what we want to know about the relations between TMV and the nonvirus proteins.

Although there are a number of extremely interesting points which I shall want to write you about, this letter will be restricted to the immediate issue of the paper which you have prepared on your results with B8. After reading your manuscript it appears evident that a good deal of confusion now exists with regard to the abnormal proteins related to TMV. The facts as I see them are these.

- 1) TMV-infected plants produce at least two and probably three low-molecular weight abnormal nonvirus proteins which are immuno-chemically related to TMV and which polymerize to form TMV-like rods.
- 2) As noted in my revision of your manuscript, there is agreement on the existence of at least two of the above proteins between myself, Jeener, and Takahashi's <u>original</u> publication (Amer. Jour. of Botany, <u>40</u>, 85). The correlations between my terminology and Takahashi's, with respect to the two proteins, is presented in my revision of your manuscript.
- 3) Very recently the paper by Delwiche, Takahashi, Newmark, et al. (Biochim. Biophys. Acta 16, 127) claims that only a single abnormal protein can be found. However, they offer no data to substantiate this claim and the method of preparation which they use is identical with one of the methods which we have employed to isolate two separate non-virus components (B3 and B6). Since seeing the Delwiche paper I have been concerned over this discrepancy and have examined the behavior of mixtures of B3 and B6. We find that such mixtures polymerize to form a single high-molecular weight component which migrates in a homogeneous fashion in the Tselius apparatus at pH 7.0. In other words, the two components will form a co-polymer. Our own preparations of B8 are made by first preparing a homogeneous preparation of B3 and then polymerizing it to form B8. Unless B3 and B6 are separated before polymerization is induced it is likely that a complex co-polymer will be formed which is difficult to distinguish from B8. I suspect very

strongly that the protein described by Delwiche et al. and which was used by Rich in his work was very likely such a co-polymer containing both B3 and B6 in an indefinite ratio.

4) For these reasons the "abnormal protein" of Rich and Delwiche should not be considered the equivalent of B8. Furthermore, if these workers, including Takahashi, are not aware of the occurrence of B6 and its participation in the formation of a co-polymer, it is entirely likely that successive preparations from their laboratories may contain variable ratios of B3 and B6. Thus, the preparation which Takahashi has sent you may contain little or no B6 and is, therefore, equivalent to our B8. We find that B6 is readily denatured when extracts are manipulated and it is possible to lose most of the B6 in a preparation as a result of lengthy processing. Hence, I would expect that the major variation in the preparations produced by the other laboratories would be a fluctuation in the relative amount of B6 present in the polymerized "abnormal protein" and that in some cases the B6 content may be zero without their being aware of it.

I have tried to incorporate some of these ideas in an addition to your manuscript. What I have added is probably unduly long and I hope that you will feel free to condense it as far as possible. I have also made a few minor changes in the first page or two of your manuscript to avoid statements which cannot be supported by our own data. This includes Richs' assertion that the chemical composition of the abnormal protein and TMV are identical. This is a very doubtful statement in view of the likelihood that their protein is not of constant composition.

Because of the material which I have added to the manuscript I think that it would be wise if my name were added to the paper. The results are extremely interesting and I am happy for the opportunity to be associated with the publication.

Also included herewith are galley proofs of two papers on the nonvirus proteins which will appear in the March 20th issue of the JOURNAL OF GENERAL PHYSIOLOGY. I am sending you these now so that you can have some idea of the evidence which we now have on the walking and properties of the nonvirus proteins.

Under separate cover I am sending you about 10 milligrams more of the B8 preparation which you now have. After some trials I decided that the best orientated preparation appeared to be a pellet of the protein spun down in the ultracentrifuge. The material is being sent to you in this form. I hope that you will be able to get an improvement in particle alignment by forcing this material into a capillary. In any event, the pellet which I am sending you is the most concentrated form in which I can prepare B8 and should make your manipulations easier to carry out.

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We are very much interested in obtaining good amino acid analyses on this preparation of B8. Consequently, I should like to ask you to try to salvage as much material as you can from your operations and return it to me when it is no longer useful to you. Regardless of the physical state of the preparation we can use it for amino acid analysis.

I hope you will write again soon and let me know what you think of the situation. In a little while I shall write you concerning some of the ideas which I have on the possible relations between the abnormal polymerized proteins and TMV nucleoprotein. Meanwhile, I shall look forward with interest to learning of your latest results.

Barry Commoner

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